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THE DIFFERENTIATION OF THE PARATYPHOID-ENTERITIDIS GROUP. I*

EDWIN O. JORDAN

From the Department of Hygiene and Bacteriology of the University of Chicago

Study of the large group of bacteria represented by *B. paratyphosus*, *B. enteritidis* (Gärtner's bacillus), and *B. suipestifer* has not yet resulted in a unified opinion. The relative importance of diagnostic characters, the cultural and pathogenic interrelationships between the different members of the group, the extent to which variation occurs, and the distribution of the several types in nature are questions on which there is no general agreement. Some observers hold that the bacillus associated with many cases of human paratyphoid fever (*B. paratyphosus* B) cannot be distinguished from a similar bacillus found in certain epidemics of meat poisoning or from the bacillus commonly present in the bodies of swine dying from hog cholera (*B. suipestifer*) (Sobernheim;¹ Weber and Haendel²). Others maintain that a clear differentiation into two types is possible on the basis of certain fermentative or serologic tests and that such a distinction corresponds with the known facts of epidemiology and distribution (Bainbridge,³ Savage⁴). On the practical side it is important to know whether human paratyphoid fever is due to infection from human sources (like typhoid fever) or from the lower animals (as is apparently true of certain 'food-poisoning' outbreaks). These considerations emphasize the necessity for better methods of differential diagnosis or failing that, a more complete knowledge of the extent and frequency of significant variation.

With this end in view, I have made a comparative study of a considerable number of microorganisms belonging to this group, and have attempted especially to determine possible correlations between agglutination reactions and differences in fermentative power and other biologic qualities. The cultures have been kept under observation for some time and tested at frequent intervals. All those here considered are of human or porcine origin. A number of them have been in my possession several years, while others are recently isolated.

* Received for publication December 5, 1916.

¹ Hyg. Rundschau, 1912, 22, pp. 953, 1019.

² Berl. klin. Wehnschr., 1912, 49, p. 2205.

³ Lancet, 1912, 1, p. 705.

⁴ Jour. Hyg., 1912, 12, p. 1.

The cultures have been obtained from various sources and I am indebted to many friends and correspondents for their courtesy. In such studies I believe that especial importance attaches to those cultures whose source and date of isolation can be exactly ascertained.

The methods used, where not otherwise stated, have conformed to the 'Standard Methods' of the American Public Health Association. The cultures received have been subjected to the following routine procedure: (a) Plating on Endo medium. (b) If colonies are all alike and conform to the paratyphoid type, 2 are transferred to agar. (c) The three strains—the original culture, and the two from single colonies—are inoculated into dextrose and lactose broth, litmus milk, and gelatin and tested for indol-production. (d) Only those strains are included in this study which, in the media mentioned, yield reactions that are typical and uniform.

The following table summarizes the list of cultures studied. Complete details as to source of isolation are given in the lists at the end of this paper.

Cultures from human sources:

- (A1) Stock cultures from laboratory collections: 4, 11, 48, 229.
- (A2) Isolated from blood in human paratyphoid fever: 3, 9, 131, 158, 188, 191, 198.
- (A3) Isolated from feces in human paratyphoid fever: 212, 213, 214, 215, 216, 217, 218, 219, 230.
- (B1) Stock cultures from laboratory collections: 2, 5, 223.
- (B2) Isolated from blood in human paratyphoid fever: 12, 130, 203, 209, 210.
- (B3) Isolated from feces in human paratyphoid fever: 47, 149, 150, 179, 211, 221,* 225.
- (B4) Isolated from human gallbladder: 8, 202.
- (B5) Isolated from organs in fatal cases of food poisoning: 151, 152, 222, 224.
- (B6) Isolated from lymph-gland infection in man: 185.
- (E1) Stock cultures from laboratory collections: 50, 51, 52.
- (E2) Isolated from human feces: 53, 206.
- (E3) Isolated from organs in fatal case of food poisoning: 228.

Culture from food materials implicated in food-poisoning outbreak: B 180.

* From a 'carrier.'

Cultures from diseased swine:

- (S1) Stock cultures of 'hog-cholera bacilli' from various laboratory collections, exact sources and date of isolation unknown: 62, 114, 115, 160, 161.
- (S2) From organs of diseased swine; full information regarding isolation: 63, 118, 132, 133, 134, 162, 163, 165, 167, 168, 169, 170, 171, 172, 173, 174, 175, 177, 178, 234.

In addition to the strains here enumerated, many stock cultures have been sent me which from the histories given seem to be from the same original source. If the reactions were identical, the duplicate cultures were discarded, and the results do not appear in these tabulations. Thus several cultures, apparently subcultures of No. 5 and identical with it, were sent me from different laboratories; only No. 5 is referred to in this paper. Fourteen strains from the same case as No. 12, isolated from blood, bile, and feces at different times gave identical reactions with it, and are not included in the final tabulation here presented. On the other hand, Nos. 212-219, tho isolated from different cases, are all from a single paratyphoid-A epidemic and are very likely all offshoots of the strain causing the outbreak. In all, about 200 cultures have been studied.

Three groups of the cultures are from human sources. These are *B. paratyphosus* A (20 strains), *B. enteritidis*, Gärtner (6 strains), and *B. paratyphosus* B (22 strains). In addition I have had under observation 1 culture (para-B type) from food material implicated in food poisoning and 25 from diseased swine, mostly from fatal 'hog-cholera' cases. These 74 cultures are alike in some of their cultural characters, but divergent in others. All are motile, gram-negative bacilli. Their growth on agar is more luxuriant as a rule than that of the typhoid bacilli, but less luxuriant than that of *B. coli*. The colonies on Endo plates vary considerably in size, and some strains show much more pink color than others. Gelatin is not liquefied by any of the strains in a 6-weeks' test.

LITMUS MILK

Milk or whey tinged with litmus has long been recognized as a useful medium in the differentiation of this group. Inoculation with strains of *B. paratyphosus* B, *B. enteritidis*, and *B. suipestifer*, at 37 C., is followed by an initial acidity which may last 2 or 3 days or longer, but which then gradually changes to an alkaline reaction. Strains of

B. paratyphosus A, on the other hand, give rise to an acid reaction which as a rule is much more persistent. Continued acid reaction has been commonly regarded as the chief cultural character separating *B. paratyphosus* A from the other groups.

Some European workers have preferred to use litmus whey rather than litmus milk for this test, but the latter is more easily prepared, and in comparative tests I have never been able to observe that whey presents any advantages over the whole milk. This is also the experience of other observers.⁵ The medium I have employed is certified milk allowed to stand and separated from the cream by siphon; to this, 7% of a 1% solution of Merck's litmus is added. The tubes so prepared are heated in the Arnold sterilizer for 20 minutes on 3 consecutive days.

The results are as follows: The freshly isolated cultures of *B. paratyphosus* B, *B. suipestifer*, and *B. enteritidis* produce at first a slight acidity, but this soon gives way—usually within 4 days—to an alkaline reaction, which increases in intensity for a week or more. Strains that have been long under cultivation often show a slower rate of increase in alkalinity. Considerable 'irregularity' is observed in certain strains.

The *paratyphosus*-A cultures remain acid as a rule much longer than those of the other group. Some writers⁶ have referred to the acidity as 'permanent,' but fail to state the exact period of observation. A number of investigators,⁷ on the other hand, have recognized that alkali-production takes place in some cultures of the A type, albeit much later than in the other group. Bainbridge says: "*B. paratyphoid* A as a rule produces permanent acidity in litmus milk, though occasionally milk may become alkaline after the lapse of several weeks." Bradley especially emphasizes the fact that "the subdivision into A and B types [on the basis of milk reaction] is one of degree, and cannot be maintained biochemically." Krumwiede and his coadjutors also record observations showing that *paratyphoid*-A strains sooner or later produce alkali in milk, and express the opinion that behavior in this medium cannot be strictly used as a qualitative method of differentiation.

⁵ Buxton, *Jour. Med. Research*, 1902, 3, p. 201. Krumwiede, Pratt, and Kohn, *ibid.*, 1916, 30, p. 55.

⁶ See, for example, J. Henderson Smith, *Brit. Med. Jour.*, 1915, 2, p. 1.

⁷ Libman, *Jour. Med. Research*, 1902, 3, p. 168. Buxton, *ibid.*, p. 201. Boycott, *Jour. Hyg.*, 1906, 6, p. 33. Bainbridge, *Jour. Path. and Bacteriol.*, 1909, 13, p. 443. Bradley, *Jour. and Proc. Roy. Soc. N. S. Wales*, 1912, 46, p. 74. Krumwiede, Pratt, and Kohn, *Jour. Med. Research*, 1916, 30, p. 53.

My own observations show that there are considerable differences in the speed and degree of alkali-formation among the members of the paratyphoid-B-enteritidis-suipestifer group, so that a series of cultures may be arranged in gradation. All the cultures of *B. paratyphosus* A in my possession have produced alkali in litmus milk after varying periods of time. Every strain has been tested in this medium, but only one series need be reported in detail here, as it is entirely typical of the whole collection.

TABLE 1
REACTION OF STRAINS OF THE PARATYPHOID-B-ENTERITIDIS-SUIPESTIFER GROUP IN
LITMUS MILK

| Days | More Acid Than Control | | Like Control | | More Alkaline Than Control | |
|------|------------------------------------|----------------|--------------------------|----------------|---|----------------|
| | Type | No. of Strains | Type | No. of Strains | Type | No. of Strains |
| 2 | Para A Para B B. enteritidis | 8 16 5 | Para B B. enteritidis | 1 3 | | 0 |
| 4 | Para A | 8 | Para B B. enteritidis | 1 1 | Para B B. enteritidis | 16 7 |
| 7 | Para A | 8 | | 0 | Para B B. enteritidis | 17 8 |
| 14 | Para A | 2 | Para A | 6 | Para B B. enteritidis | 17 8 |
| 30 | Para A | 1 | | | All others more alkaline than control | |

Arranged in order of increasing alkalinity, changes are observed in the relative position of cultures (all kept in the same rack at 37 C. under identical conditions). If the strains are recorded in gradation — No. 1 representing the most acid, and No. 33 the most alkaline, after 4 days' growth — the order after 7 days is as follows: A3, A7, A1, A5, A2, A8, A6, A4, B11, B13, B10, B12, E9, B20, B16, E23, E15, B14, B22, E25, B19, B27, B21, B24, E30, E17, E28, E32, B18, B26, B31, E33, B29; in 14 days: A7, A1, A2, A3, A8, A4, A6, A5, B11, B10, E9, B12, B13, B14, E23, E15, B21, B27, E25, B24, E30, B22, E17, E28, B16, B20, B19, E32, B29, E33, B26, B18, B31; and in 30 days: A1, A8, A6, A7, A5, A2, A4, A3, B11, E15, B10, E32, B21, B12, B27, B14, E28, E30, E23, B13, E9, E25, B24, E17, E33, B18, B31, B16, B22, B29, B20, B19, B26.

The sort of deviation observed in a series of paratyphoid-A strains is shown in Table 2. Alkali-formation is first observable after about

2 weeks and then proceeds much more rapidly in some strains than in others. There is often a difference in the amount and rapidity of alkali-formation in strains obtained from single colonies after plating an apparently pure culture on Endo medium (see Nos. 215 and 219, Table 2).

TABLE 2

| Strain | Reaction in Litmus Milk after | | | | | |
|---------|-------------------------------|----------|--------|---------|---------|---------|
| | 24 Hours | 48 Hours | 5 Days | 11 Days | 15 Days | 30 Days |
| 212 | A | A | A | A | S A | Alk |
| 212 C1 | A | A | A | A | S A | Alk |
| 212 C2 | A | A | A | A | S A | Alk |
| 213 | A | A | A | A | S A | Alk |
| 213 C1 | A | A | A | A | S A | Alk |
| 213 C2 | A | A | A | A | S A | Alk |
| 214 | A | A | A | A | S A | Alk |
| 214 C1 | A | A | A | A | S A | Alk |
| 214 C2 | A | A | A | A | S A | Alk† |
| 215 | A | A | A | A | S A | Alk |
| 215 C1 | A | A | A | A | S A | Alk |
| 215 C2 | A | A | A | A | C | Alk† |
| 216 | A | A | A | A | S A | Alk† |
| 216 C1 | A | A | A | A | S A | Alk† |
| 216 C2 | A | A | A | A | S A | Alk† |
| 217 | A | A | A | A | S A | Alk |
| 217 C1 | A | A | A | A | S A | Alk |
| 217 C2 | A | A | A | A | S A | Alk |
| 218 | A | A | A | A | S A | Alk |
| 218 C1 | A | A | A | A | S A | Alk |
| 218 C2 | A | A | A | A | S A | Alk |
| 219 | A | A | A | A | S A | Alk |
| 219 C1* | A | A | A | A | S A | Alk† |
| 219 C2 | A | A | A | A | S A | Alk |

A = more acid than control.

S A = slightly acid.

C = like control.

Alk = more alkaline than control.

* 219 C1 decidedly more alkaline in 20 days than any other.

† Decidedly more alkaline than others, which are all approximately the same.

A comparison has been made between the rate of alkali-formation in litmus milk in a shallow layer in Erlenmeyer flasks (0.5 cm.) and that in test tubes one-half to two-thirds filled (about 8 cm.). There is sometimes a slight acceleration of alkali-production in the former as compared with that in the deep tubes, but many strains maintain a persistent acid reaction in the flasks for 2 weeks. The difference between tubes and flasks in this respect is not as great as was anticipated.

In other series like that recorded in Table 1, and including all the cultures mentioned in this paper, similar results have been obtained. The strains of *B. paratyphosus* B (22 strains*), *B. enteritidis* (6 strains), and *B. supeptifer* (25 strains) have all begun to show alka-

* One of these strains, 221, has produced alkali much more slowly than the others and shows beginning alkalinity only after about 10 to 14 days. Even then, however, it can be distinguished from all the A strains I have had under observation and in 20 days the difference is still more marked.

linity earlier than any of the 20 strains of *B. paratyphosus* A. The latter without exception have remained more acid than the control tube for nearly 2 weeks, but in about 14 to 15 days (at 37 C.) many of these approximate the reaction of the control and then become progressively alkaline. The alkalinity of all the A strains at the end of 5 weeks is decided, but is less than that of the others, so that in a rack with 50 to 100 tubes the A milk tubes can be readily distinguished. For the practical differentiation of the A type litmus milk is of the highest value, tho taken by itself the test is probably not absolutely differential any more than any other single test, especially in dealing with old stock cultures that have begun to vary.

INDOL

The early statements regarding indol-production by members of this group are conflicting, the result probably of the diversity of methods used.⁸ Since the introduction of the delicate and accurate paradimethyl-amido-benzaldehyde reaction, most observers have agreed that strains giving a positive indol reaction are at least exceedingly rare. I have tested over 200 strains from various sources and have found a positive reaction only in those that show a distinct difference from the true paratyphoid types either biochemically or by agglutination tests.

The indol tests have been made both in the standard peptone medium (paradimethyl-amido-benzaldehyde reaction) and also in most instances by the rapid and convenient modification of Zipfel's tryptophane medium devised by Cannon.⁹ The 74 strains of *B. paratyphosus* A, *B. paratyphosus* B, *B. enteritidis*, and *B. suipestifer* described in this paper have all given consistently negative results. It seems fair to conclude that indol-production by the members of this group is at least as rare as it is among typhoid bacilli.

CARBOHYDRATE-FERMENTATION

The fermentation reactions shown by members of the paratyphoid-enteritidis group have been the subject of extensive study by many investigators, and have constituted the ordinary means of distinguishing

⁸ Kolle and Wassermann, *Handb. d. pathogen. Mikroorganismen*, 1913, 3, p. 1017.

⁹ *Jour. Bacteriol.*, 1916, 1, p. 535.

these organisms from *B. typhosus* and *B. coli*. As well known, the typical carbohydrate reaction (acid- and gas-production) of the three groups is as follows:

| TYPE | DEXTROSE | LACTOSE | SACCHAROSE |
|------------------------------|----------|---------|------------|
| <i>B. typhosus</i> | — | — | — |
| <i>B. paratyphosus</i> | + | — | — |
| <i>B. coli</i> | + | + | ± |

Only 1 strain among the 74 under observation has failed to conform to this typical classification. This is No. 134, isolated by Dorset in 1899 from the spleen of a pig dying from "acute hog cholera" in Page County, Iowa.¹⁰ This organism resembles the typhoid bacillus in producing acid but no gas in dextrose broth. My own tests, made on this organism from 14 to 16 years after its isolation, show that no change has occurred in this particular. Other characteristics of this bacillus, including some of great diagnostic value, relate it to the paratyphoid-enteritidis group. Similar strains of *B. suis* which do not produce gas in dextrose and other carbohydrate media, but are indistinguishable from the types in other respects, have been isolated by Bock,¹¹ Bainbridge,¹² and others. TenBroeck¹³ has found a non-gas-producing strain in an old stock culture of hog-cholera bacillus that had been under cultivation for 14 years. It resembles the typical hog-cholera bacillus in agglutination and other characters, but fails to form gas in the carbohydrates usually attacked by this organism.

With the exception of No. 134 all the cultures listed agree in their fermentative behavior (gas-production) toward dextrose (+), lactose (—), and saccharose (—).*

The differential diagnosis may be made quite simply by the use of standard nutrient broth tinged with litmus and containing 0.5% of the carbohydrate to be tested. I have also used the Barsiekow media (litmus-nutrose-dextrose and litmus-nutrose-lactose) employed by some German investigators, but so far as my experience goes they afford no additional information in the differentiation of this group.

¹⁰ 18th Ann. Rep., Bur. An. Ind., 1901, p. 566.

¹¹ Arb. a. d. k. Gesndtsamte, 1906, 24, p. 238.

¹² Jour. Path. and Bacteriol., 1908-9, 13, p. 443.

¹³ Jour. Exper. Med., 1916, 24, p. 213.

* As pointed out by several investigators, certain sources of error in such tests must be guarded against. It is essential to obtain carbohydrates quite free from dextrose. If meat extract or meat infusion is used, it must be free from muscle sugar. When sodium hydrate is used for neutralization, a small bubble of gas may sometimes develop in the culture. Further, unless overheating is avoided in the sterilization of lactose broth, particularly if the reaction is alkaline, a small amount of dextrose will be formed, the presence of which will falsify the fermentation reaction.

A large number of other carbohydrates have been tested by various observers in the study of fermentative characteristics. There is practically general agreement on the results summarized in the following table:

TABLE 3
CARBOHYDRATE-FERMENTATION (ACID- AND GAS-PRODUCTION) BY PARATYPHOID-ENTERITIDIS
BACILLI

| + | — | ± |
|---|--|---------------------------------|
| Rhamnose Dextrose Galactose Mannose Levulose Maltose* Mannite Sorbitol | Lactose Saccharose Raffinose Dextrin Inulin Erythrite Adonite Salicin | Arabinose Xylose Dulcitol |

* Nearly all observers have reported gas- and acid-production in maltose, but May (Jour. Trop. Med. and Hyg., 1911, 14, p. 1) states that only acid was produced in the strain isolated by him from a water supply. Proescher and Roddy (Arch. Int. Med., 1910, 5, p. 263) state that "maltose is not fermented by Para A, but some cultures of Para B cause slight fermentation." In my own series all cultures (except 134) including the para-A strains, have fermented maltose, with gas-production.

There are 3 carbohydrates in this list that are not fermented uniformly by all the members of these groups, namely dulcitol, arabinose, and xylose. It is a disputed question whether such differences in fermentative power are correlated with other characters and whether they permit a differentiation into subgroups of biologic or practical importance.

DULCITE

Positive dulcitol-fermentation was noted by Voges and Proskauer¹⁴ for certain strains of hog-cholera bacilli and by Conradi, Drigalski, and Jürgens¹⁵ for certain paratyphoid organisms. Kligler¹⁶ has attempted to distinguish between *B. suipestifer* (regarded as dulcitol—) and *B. enteritidis* (dulcitol+) on the basis of dulcitol-fermentation. Ford,¹⁷ on the contrary, found that 5 cultures of "the bacillus of hog cholera" agreed in fermenting dulcitol.

The majority of investigators have observed acid- and gas-production in dulcitol by the organisms of this group. This is the case with the strains studied by Morgan,¹⁸ Sacquépée and Chevrel,¹⁹ Boycott,²⁰ MacConkey,²¹ Savage and

¹⁴ Ztschr. f. Hyg. u. Infektionskr., 1898, 28, p. 20.

¹⁵ Ibid., 1903; 42, p. 141.

¹⁶ Jour. Infect. Dis., 1914, 15, p. 187.

¹⁷ Med. News, 1905, 86, p. 1126.

¹⁸ Brit. Med. Jour., 1905, 1, p. 1257.

¹⁹ Ann. de l'Inst. Pasteur, 1906, 20, p. 1.

²⁰ Jour. Hyg., 1906, 6, p. 33.

²¹ Ibid., p. 570.

Gunson,²² Bainbridge,¹² May,²³ Bradley,²⁴ Poppe,²⁵ Seiffert,²⁶ Biewald,²⁷ and Ducamp.²⁸ Morgan, however, records negative results with one strain isolated from hog cholera, and his observation was confirmed for the same culture by Savage,²⁹ who, however, notes that another strain from hog cholera reported by Morgan as giving positive fermentation was negative in his own hands. Ducamp observed that 2 hog cholera strains tested by him did not attack dulcité. Bradley²⁴ found a difference in the rapidity with which the fermentation is effected: "On dulcité acid and gas are produced by all the strains (40 stock cultures principally from European laboratories), but the time taken is usually longer than for the other sugars and is especially long with the hog cholera group." Robinson³⁰ has recorded a negative dulcité reaction for several strains of paratyphoid organisms isolated by him from the feces of 3 persons infected during a water-borne epidemic. One* of these (179 = Robinson's No. 16.3) has always fermented dulcité promptly (within 24 hours) since it first came into my hands about 2 years after isolation. When the discrepancy with Robinson's results was noted, the culture was plated and 12 colonies picked. These 12 strains inoculated into dulcité gave identical reactions, acid and gas in 24 hours. Proescher and Roddy³¹ state that "dulcité is slightly fermented by 12 per cent. of the cultures"—mostly from human paratyphoid cases—which they examined.

In my own observations the following strains have fermented dulcité within 24 hours with acid- and gas-production: 2, 5, 8, 12, 47, 130, 149, 150, 151, 152, 179, 180, 185, 202, 203, 209, 210, 211, 221, 223, 225, 62, 115, 161, [169]; the following have failed to ferment dulcité in 24 hours, but have produced some gas in 5 days (168, 173, 174) (10 days, 172); the following have shown no gas or acid in 15 days: 63, 114, 118, 132, 133, 160, 162, 163, 165, 167, 170, 171, 175, 177, 178.

It thus appears that all 22 of the cultures from human sources ferment dulcité promptly, while only 4 of 25 of the cultures from swine ferment dulcité within 24 hours. Five other strains ferment dulcité tardily (within 15 days). Furthermore, 3 of the porcine cultures giving a prompt positive reaction with dulcité were received from various laboratory collections under the name of *B. cholera-suis*, *B. suipestifer*, or *bacillus* of hog cholera, and their precise origin is

²² *Ibid.*, 1908, 8, p. 601.

²³ *Jour. Trop. Med. and Hyg.*, 1911, 14, p. 1.

²⁴ *Jour. Proc. Roy. Soc. N. S. Wales*, 1912, 46, p. 74.

²⁵ *Ztschr. f. Infektionskr. d. Haust.*, 1908-9, 5, p. 42. Quoted by Hübener, *Fleischvergiftungen u. Paratyphusinfektionen*, Monographie, 1910, p. 78.

²⁶ *Ztschr. f. Hyg. u. Infektionskr.*, 1909, 63, p. 273.

²⁷ *Inaug. Diss. Giessen*, 1909; cited by Hübener,²⁵ p. 80.

²⁸ *These*, Lille, 1907; cited by Hübener,²⁵ p. 80.

²⁹ *Rep. Med. Officer to Local Gov't Board*, 1907-8, p. 425.

³⁰ *Jour. Infect. Dis.*, 1915, 16, p. 448.

* This strain is said to have produced "permanent acidity" in litmus milk, but ever since it came into my hands it has given a typical paratyphus-B reaction (definite alkalinity within 5 days) and has shown no sign of variation within a year tho frequently plated out and tested by transfer of individual colonies.

³¹ *Arch. Int. Med.*, 1910, 5, p. 263.

unknown. Included in this number are 2 cultures from European laboratories: 115 (from Kral) and 161 (from Ostertag). Only one porcine strain (169) whose history is fully known has fermented dulcitate promptly and this was negative when first tested.* The tardy or negative dulcitate-fermentation here manifested is correlated, as will appear, with differences in arabinose-fermentation and agglutination reactions. As already noted, such differences in dulcitate-fermentation as are recorded by previous observers suggest a negative or relatively little dulcitate-fermentation by members of the 'hog-cholera' group (Morgan,¹⁸ Savage,²² Bradley,²⁴ Ducamp,²⁸ Kligler¹⁶).

All 20 strains of *B. paratyphosus*-A type agree in attacking dulcitate tardily. There is no acid or gas formed in any case within 24 hours and in the case of only 3 strains (131, 188, 191) within 48 hours. All but one (11), however, have produced gas and acid in 5 days and this late strain is positive in 10 days. This characteristic of delayed dulcitate-fermentation has not previously been noted as a differential mark. In my series of cultures it distinguishes the *B. paratyphosus*-A strains from the *B. paratyphosus*-B type, all of which (23 strains) ferment dulcitate within 24 hours. In this respect the A strains approximate somewhat to the *B. suispestifer* type, but unlike the latter they all ferment arabinose promptly—within 24 hours.

All the Gärtner strains (*B. enteritidis*) have fermented dulcitate within 24 hours, in this respect being identical with the *paratyphosus*-B strains.

ARABINOSE

The use of this carbohydrate has been frequently suggested for differential purposes.

Several observers have noted that "hog cholera strains" are particularly apt to give negative results with arabinose.³² Seiffert²⁸ found that several strains of bacilli from paratyphoid fever and meat-poisoning cases and also 2 hog-

* This strain, isolated from the lung of a hog dead from hog cholera, has undergone a definite change since it first came into my hands in June, 1915. It then failed to ferment arabinose in 14 days, and did not attack dulcitate within 48 hours, tho producing both acid and gas within 14 days. Dulcitate-fermentation within 24 hours was still negative on Dec. 2, 1915, April 20, and May 21, 1916 (3 colonies from plates), but on the last named date there was a noticeable acceleration in this respect, and a positive reaction occurred earlier (within 48 hours) than in preceding tests. On Oct. 18, 1916, the culture gave positive dulcitate-fermentation within 24 hours, as did inoculations with 3 colonies picked from an agar plate. On Nov. 10, 1916, 12 colonies picked from a plate gave identical positive results, acid and gas within 24 hours. Coincident with this change in dulcitate-fermenting powers arabinose-fermentation has become positive (acid and gas within 24 hours). Agglutination reactions have been somewhat irregular, but where absorption tests have been made the early ones were of the *suispestifer* type, the later ones of the *paratyphosus*-B type. The 12 strains isolated Nov. 10, 1916, were identical in their agglutination reactions (*paratyphoid*-B¹² serum saturated with *suispestifer* 170) with the typical *paratyphoid*-B strains.

³² Ford, Med. News, 1905, 86, p. 1126. Bradley, Proc. Roy. Soc. N. S. Wales, 1912, 46, p. 74.

cholera strains (sources not given) fermented arabinose. Langkau³³ observed that 6 strains from calf diarrhea did not ferment arabinose, while others of various origin, including one "Schweinepest" strain, were positive.³⁴ The results reported by others³⁵ do not show complete uniformity; full data are not given in all cases respecting the sources of the cultures tested.

All the strains of the para-B type of human origin in my possession ferment arabinose promptly. On the other hand, only 4 of 25 of the cultures from swine produced gas in arabinose within 24 hours. These are the same strains that ferment dulcitol either slowly or not at all. There is thus an exact correlation in this group of cultures. Those cultures that ferment dulcitol and arabinose promptly are of human origin (23 strains), while the majority of those attacking these carbohydrates tardily or not at all are porcine strains (21 of 25).

XYLOSE

Xylose like arabinose is not fermented uniformly by all strains.

Ford¹⁷ found that some stock "paratyphoid" cultures fermented xylose while others did not. The "hog-cholera" strains that he tested all failed to attack this carbohydrate. Seiffert²⁶ noted a positive result with the strains in his hands as did Biewald.²⁷ Langkau³³ observed that bacilli from calf-diarrhea produced no gas in xylose in the first 24 hours, but could not be distinguished from the other strains after 48 hours. Ducamp²⁸ observed that 2 hog-cholera strains did not attack xylose. Proescher and Roddy³¹ state that "Xylose is fermented by very few cultures of A and B; most of them cause no fermentation." Schern's results have already been mentioned.

An important differential result was obtained by Harding and Osterberg.³⁰ These investigators, working with fuchsin sulfite media (Endo) found that acid was produced in the presence of xylose by 12 paratyphoid-B and enteritidis strains but not by 1 mouse-typhoid and 5 paratyphoid-A strains. Their results were confirmed and extended by Krumwiede and his coadjutors,³⁷ who found that the 20 para-A strains in their hands failed to ferment xylose while the other members of the paratyphoid-enteritidis group gave positive results. One "hog-cholera" strain, however, fermented only slowly. Apparently only the change in reaction was noted by these investigators, no statement being made as to gas-production.

³³ Inaug. Diss., Leipzig, 1909; cited by Hübener,²⁸ p. 80.

³⁴ Schern's observations (Arb. a.d.k. Gsndhtsamte, 1909-10, 33, p. 387) on the behavior of various strains of paratyphoid and hog-cholera bacilli relate to the color changes produced in arabinose and xylose broth tintured with litmus. Altho he notes differences in the strains of human and animal origin, these are not universal, and no attempt is made to correlate them with other characters. It is not apparent from Schern's article whether the differences recorded for various strains—"red," "yellow," "violet," etc.—are constant for each strain under slightly varying conditions of experimentation or over any considerable period of time.

³⁵ Bahr, Raebiger, and Grosso, *Ztschr. f. Infektionskr. d. Haust.*, 1909, 5, p. 295. Bainbridge, *Jour. Path. and Bacteriol.*, 1909, 13, p. 443. Proescher and Roddy, *Arch. Int. Med.*, 1910, 5, p. 263.

³⁶ *Jour. Infect. Dis.*, 1912, 11, p. 109.

³⁷ Krumwiede, Pratt, and Kohn, *Jour. Med. Research*, 1916, 29, p. 355.

In the series with which I have worked, the paratyphoid-A strains are marked off sharply from the others by their inability to ferment xylose. My observations on this point were made for the most part in June, 1915, and are in quite complete accord with those of Harding and Ostenberg and Krumweide, Pratt, and Kohn. None of the paratyphoid-A strains produce either gas or acid in xylose broth. All the other strains here considered produce gas except 134 (atypical suipestifer strain, forming no gas in dextrose) and 153 (*B. enteritidis*). Both these strains, however, produce acid. No. 115 ("Bacillus of hog cholera," Kral's collection) produces acid vigorously, but gas very slowly.

AGGLUTINATION

Rabbits have been used for the agglutination tests. Subcutaneous inoculations of killed bacilli, washed from agar slants, have first been given, followed by intraperitoneal injections of living bacilli. After 4 or 5 intraperitoneal injections the titer of the serum is usually as high as 5000 to 10000, tho there are marked differences in the agglutino-genic qualities of different strains. A high titer—30000 to 40000—has been reached more readily with *B. enteritidis* strains than with the others used for this purpose.

The macroscopic method has been used throughout. All suspensions and dilutions were made with sterile salt solution. Fresh 24-hour-old agar cultures have always been employed. The mixture of serum and bacterial suspensions has been inoculated 2 hours at 37 C., then left overnight, about 18 hours, in the refrigerator and the reading taken. In absorption tests mixtures of serum and absorbing bacilli have been incubated 1 hour at 37 C. before centrifugating.

Four distinct divisions based on agglutinability are recognizable in the organisms here considered: (1) *B. paratyphosus* A, (2) *B. enteritidis*, (3) *B. paratyphosus* B, and (4) *B. suipestifer*.

(1) *B. paratyphosus* A.—Serum produced by organisms of *B. paratyphosus*-A type has agglutinated organisms of the same group to practically the same dilutions as the homologous strains. Table 4 is representative of the results obtained with this serum. All cultures have been tested with this serum, but since the results are similar, it does not seem necessary to tabulate them in detail. Strains 212-219 resemble 191 in showing relatively slight agglutinability in the lower dilutions (1:250), but show a trace of agglutination at 1:5000 as definitely as the homologous strains. As a rule the members of the

other three groups do not show a trace of agglutination at 1:250 or even 1:100 with B.-paratyphosus-A serum. A few anomalous strains, usually stock cultures long under cultivation, exhibit mixed or uncertain agglutinative affinities. These irregular strains will be considered in a later article.

TABLE 4
AGGLUTINATION WITH B.-PARATYPHOSUS-A SERUM (4)

| Strain | 1:250 | 1:500 | 1:1000 | 1:2000 | 1:5000 | 1:10000 |
|-------------------|-------|-------|--------|--------|--------|---------|
| 3 | ++ | + | + | tr | tr | 0 |
| 4 | ++ | ++ | + | + | tr | 0 |
| 9 | +++ | ++ | ++ | + | 0 | 0 |
| 48 | ++ | ++ | + | + | tr | 0 |
| 131 | ++ | + | + | + | tr | 0 |
| 158 | +++ | ++ | + | + | tr | 0 |
| 188 | ++ | ++ | ++ | + | tr | 0 |
| 191 | + | + | + | + | tr | 0 |
| 198 | ++ | ++ | ++ | + | 0 | 0 |
| 152 | 0 | 0 | 0 | 0 | 0 | 0 |
| B. paratyphosus B | | | | | | |
| 206 | 0 | 0 | 0 | 0 | 0 | 0 |
| B. enteritidis | | | | | | |
| 177 | 0 | 0 | 0 | 0 | 0 | 0 |
| B. suispestifer | | | | | | |

+++ = complete
++ = marked
+ = slight
tr = trace
0 = none

(2) *B. enteritidis*.—Serum produced by organisms of *B. enteritidis* type (52) agglutinates the other strains of this group in high dilutions (1:30000), but in only 2 instances has it affected organisms of the *suispestifer*, *paratyphosus-B*, or *paratyphosus-A* groups in a dilution of 1:100 and never in that of 1:250. Conversely the 6 strains of *B. enteritidis* under observation are agglutinated slightly or not at all by highly potent sera of the other groups (Table 5).

TABLE 5

| Enteritidis Strain | Suispestifer Serum (118) Titer, 1:5000 | Paratyphosus B Serum (12) Titer, 1:10000 | Paratyphosus A Serum (4) Titer, 1:5000 |
|--------------------|--|--|--|
| 50 | <100 | <250 | 500 |
| 51 | <100 | <250 | 100 |
| 52 | <100 | <250 | 100 |
| 53 | <100 | <250 | 250 |
| 228 | <100 | <250 | 100 |
| 206 | <100 | <250 | 250 |

(3) *B. paratyphosus B*.—The agglutinating sera produced by various *paratyphosus-B* strains (2, 5, 12) have given substantially the same results. All have agglutinated the other strains of the

B.-paratyphosus-B group in relatively high dilutions. Many strains of B.-suipestifer are also agglutinated in practically the same dilution by the paratyphosus-B sera, but the paratyphosus-A strains as a rule are not affected by highly potent sera. The B.-enteritidis strains are often slightly agglutinated. One typical series is given in Table 6.

TABLE 6
PARA-B SERUM (5)*

| Para B | | B. Suipestifer | | Para A | | B. Enteritidis | |
|--------|---------------|----------------|---------------|--------|---------------|----------------|---------------|
| No. | Agglutination | No. | Agglutination | No. | Agglutination | No. | Agglutination |
| 2 | 5000 + | 62 | 5000 + | 3 | <100 | 50 | 250 + |
| 5 | 5000 ++ | 63 | 1000 + | 4 | <100 | 51 | 250 + |
| 8 | 5000 +++ | 114 | 1000 + | 9 | <100 | 52 | 250 + |
| 12 | 2000 ++ | 115 | 5000 ++ | 11 | <100 | 53 | <250 |
| 47 | 5000 + | 118 | 5000 + | 48 | <100 | 228 | 500 + |
| | | 132 | 250 ++ | 131 | <100 | | |
| 130 | 5000 + | 133 | 1000 ++ | 158 | <100 | | |
| 149 | 5000 + | 134 | 5000 +++ | | | | |
| 150 | 2000 ++ | 160 | 100 + | | | | |
| 151 | 5000 + | 161 | 500 ++ | | | | |
| 152 | 2000 + | 162 | 500 + | | | | |
| | | 163 | 1000 + | | | | |
| | | 165 | 1000 + | | | | |
| | | 167 | 1000 + | | | | |
| | | 168 | 500 + | | | | |
| | | 169 | 250 ++ | | | | |
| | | 170 | 100 + | | | | |
| | | 171 | 1000 + | | | | |
| | | 172 | 100 + | | | | |
| | | 173 | 100 + | | | | |
| | | 174 | 100 + | | | | |
| | | 175 | 500 + | | | | |
| | | 177 | <100 | | | | |
| | | 178 | 250 + | | | | |

+++ = complete

++ = marked

+ = slight

* B.typhosus was not agglutinated by this serum at 1:100.

The paratyphosus-B strains are also agglutinated in varying degrees with B.-suipestifer sera. One series is shown in Table 7. Often the agglutination limit is almost as high as the titer of the serum (see especially Nos. 130 and 151). Absorption tests, however, show the real nature of the agglutinative reactions. Table 8, which should be compared with Table 7, affords an excellent demonstration of the group relationship of these organisms. Variations are frequently observed. Thus No. 180 agglutinated feebly or not at all with suipestifer serum for a long time after it came into my possession, but in recent tests it shows greater agglutinability. No. 221 was highly refractory to suipestifer serum at the date of the test recorded in Table 7 (1-19-'17).

TABLE 7
B.-SUIPESTIFER (118) SERUM

| Strain | 1:250 | 1:500 | 1:1000 | 1:2000 | 1:5000 | 1:10000 |
|--------|-------|-------|--------|--------|--------|---------|
| 5 | ++ | + | + | tr | 0 | 0 |
| 8 | +++ | ++ | + | 0 | 0 | 0 |
| 12 | +++ | ++ | + | tr | 0 | 0 |
| 47 | +++ | ++ | + | 0 | 0 | 0 |
| 130 | +++ | +++ | ++ | ++ | + | tr |
| 149 | ++ | ++ | + | + | 0 | 0 |
| 150 | ++ | ++ | + | + | 0 | 0 |
| 151 | +++ | ++ | ++ | ++ | + | tr |
| 152 | tr | 0 | 0 | 0 | 0 | 0 |
| 179 | ++ | ++ | + | + | tr | 0 |
| 180 | ++ | + | + | tr | 0 | 0 |
| 185 | ++ | ++ | + | tr | 0 | 0 |
| 202 | ++ | + | + | tr | 0 | 0 |
| 203 | + | + | + | tr | 0 | 0 |
| 209 | +++ | ++ | + | + | 0 | 0 |
| 210 | ++ | + | + | tr | 0 | 0 |
| 211 | tr | tr | tr | tr | 0 | 0 |
| 221 | 0 | 0 | 0 | 0 | 0 | 0 |

+++ = complete
 ++ = marked
 + = slight
 tr = trace
 0 = none

TABLE 8
B.-SUIPESTIFER (118) SERUM SATURATED WITH B. PARATYPHOSUS B (12)

| Strain | 1:250 | 1:500 | 1:1000 | 1:2000 | 1:5000 | 1:10000 |
|--------|-------|-------|--------|--------|--------|---------|
| 5 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8 | tr | 0 | 0 | 0 | 0 | 0 |
| 12 | 0 | 0 | 0 | 0 | 0 | 0 |
| 47 | tr | 0 | 0 | 0 | 0 | 0 |
| 130 | tr | 0 | 0 | 0 | 0 | 0 |
| 149 | 0 | 0 | 0 | 0 | 0 | 0 |
| 150 | 0 | 0 | 0 | 0 | 0 | 0 |
| 151 | tr | 0 | 0 | 0 | 0 | 0 |
| 152 | 0 | 0 | 0 | 0 | 0 | 0 |
| 179 | tr | 0 | 0 | 0 | 0 | 0 |
| 180 | 0 | 0 | 0 | 0 | 0 | 0 |
| 185 | tr | 0 | 0 | 0 | 0 | 0 |
| 202 | tr | 0 | 0 | 0 | 0 | 0 |
| 203 | 0 | 0 | 0 | 0 | 0 | 0 |
| 209 | 0 | 0 | 0 | 0 | 0 | 0 |
| 210 | 0 | 0 | 0 | 0 | 0 | 0 |
| 211 | tr | 0 | 0 | 0 | 0 | 0 |
| 221 | 0 | 0 | 0 | 0 | 0 | 0 |

Table 11 shows the correlation of fermentative and agglutinative characters in the organisms of this group.

(4) *B. suipestifer*.—Agglutinating sera have been produced with various strains of porcine origin (63, 118, 167) and have given relatively uniform results. The majority of the strains of porcine origin agglutinate in as high a dilution as the homologous strains (Table 9). Saturation of the serum with a strain of *B. paratyphosus* B affects but little its agglutinative power for the typical porcine strains (Table 10).

Especial attention may be directed to Nos. 62, 115, and 161, Table 10, which are old stock strains and which, tho presumably of porcine origin, evince an agglutinative affinity to the B.-paratyphosus-B type. These three strains all ferment dulcitate and arabinose promptly and in this respect as in agglutinative reaction resemble the strains of human origin (Table 12). Two strains of porcine origin (Nos. 169 and 175) are not recorded on Tables 9 and 10. These strains, when they were first examined a few months after isolation, showed all the characteristics of what I have called the *suipestifer* type, but in the course of 18 months' cultivation they have altered, both biochemically and agglutinatively, and are now of the paratyphosus-B type in fermentative and agglutinative characters. Sometimes a *suipestifer* serum has been obtained which manifests a definite differential behavior toward the paratyphosus-B and *suipestifer* types without saturation. The action of such a serum is recorded in Table 13. The strains of the paratyphosus-B type (23 of human origin and 5 of porcine origin) are affected slightly or not at all by the *suipestifer* sera.

TABLE 9
B.-SUIPESTIFER (118) SERUM

| Strain | 1:250 | 1:500 | 1:1000 | 1:2000 | 1:5000 | 1:10000 |
|--------|-------|-------|--------|--------|--------|---------|
| 12 | ++ | ++ | ++ | + | tr | 0 |
| 62 | +++ | ++ | ++ | + | 0 | 0 |
| 63 | +++ | +++ | +++ | ++ | + | tr |
| 114 | +++ | +++ | ++ | ++ | + | tr |
| 115 | ++ | ++ | + | + | tr | 0 |
| 118 | +++ | +++ | ++ | + | tr | tr |
| 132 | +++ | +++ | ++ | ++ | + | tr |
| 133 | +++ | ++ | ++ | ++ | + | tr |
| 160 | +++ | ++ | ++ | ++ | + | — |
| 161 | ++ | ++ | ++ | ++ | + | 0 |
| 162 | +++ | +++ | ++ | ++ | + | tr |
| 163 | +++ | ++ | ++ | ++ | + | tr |
| 165 | +++ | +++ | ++ | ++ | + | tr |
| 167 | +++ | ++ | ++ | + | + | tr |
| 168 | +++ | +++ | ++ | ++ | ++ | + |
| 170 | +++ | ++ | ++ | ++ | + | tr |
| 171 | ++ | ++ | ++ | ++ | + | tr |
| 172 | +++ | ++ | ++ | ++ | + | tr |
| 173 | +++ | ++ | ++ | ++ | + | tr |
| 174 | +++ | +++ | +++ | ++ | ++ | + |
| 177 | +++ | +++ | +++ | ++ | ++ | tr |
| 178 | +++ | ++ | ++ | + | tr | tr |

+++ = complete
 ++ = marked
 + = slight
 tr = trace
 0 = none

TABLE 10
B.-SUIPESTIFER (118) SERUM SATURATED WITH B. PARATYPHOSUS B (12)

| Strain | 1:250 | 1:500 | 1:1000 | 1:2000 | 1:5000 | 1:10000 |
|--------|-------|-------|--------|--------|--------|---------|
| 12 | tr | 0 | 0 | 0 | 0 | 0 |
| 62 | + | tr | 0 | 0 | 0 | 0 |
| 63 | ++ | ++ | ++ | + | + | tr |
| 114 | ++ | ++ | ++ | + | + | 0 |
| 115 | tr | 0 | 0 | 0 | 0 | 0 |
| 118 | ++ | + | + | + | tr | 0 |
| 132 | ++ | ++ | + | + | tr | 0 |
| 133 | ++ | ++ | ++ | + | 0 | 0 |
| 160 | ++ | ++ | ++ | + | + | tr |
| 161 | tr | 0 | 0 | 0 | 0 | 0 |
| 162 | ++ | ++ | ++ | + | + | tr |
| 163 | ++ | ++ | + | + | tr | tr |
| 165 | ++ | ++ | ++ | + | + | tr |
| 167 | ++ | ++ | ++ | + | + | tr |
| 168 | ++ | ++ | ++ | ++ | + | 0 |
| 170 | ++ | ++ | ++ | ++ | + | 0 |
| 171 | ++ | ++ | ++ | ++ | + | tr |
| 172 | ++ | ++ | ++ | + | + | tr |
| 173 | ++ | ++ | ++ | ++ | + | tr |
| 174 | ++ | ++ | ++ | ++ | + | + |
| 177 | ++ | ++ | ++ | ++ | + | tr |
| 178 | ++ | ++ | ++ | ++ | + | tr |

+++ = complete
 ++ = marked
 + = slight
 tr = trace
 0 = none

TABLE 11
STRAINS OF HUMAN ORIGIN

| Strain | Rapid Dulcitol- Fermentation (within 24 hr.) | Rapid Arabinose- Fermentation (within 24 hr.) | Agglutination with B.-Paratyphosus-B No. 12 Serum | |
|--------|---|--|--|--|
| | | | Before Saturation | After Saturation with Suipestifer 167 |
| 2 | + | + | 5000 | 2000 |
| 5 | + | + | 5000 | 2000 |
| 8 | + | + | 10000 | 2000 |
| 12 | + | + | 5000 | 2000 |
| 47 | + | + | 10000 | 10000 |
| 130 | + | + | 5000 | 2000 |
| 149 | + | + | 5000 | 2000 |
| 150 | + | + | 5000 | 5000 |
| 151 | + | + | 5000 | 2000 |
| 152 | + | + | 2000 | 500 |
| 179 | + | + | 5000 | 2000 |
| 180 | + | + | 2000 | 2000 |
| 185 | + | + | 5000 | 5000 |
| 202 | + | + | 5000 | 2000 |
| 203 | + | + | 10000 | 10000 |
| 209 | + | + | 10000 | 10000 |
| 210 | + | + | 10000 | 10000 |
| 211 | + | + | 10000 | 10000 |
| 221 | + | + | 2000 | 2000 |
| 222 | + | + | 5000 | 2000 |
| 223 | + | + | 5000 | 2000 |
| 224 | + | + | 10000 | 5000 |
| 225 | + | + | 10000 | 10000 |

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TABLE 12
STRAINS OF PORCINE ORIGIN

| Strain | Rapid Dulcitol- Fermentation (within 24 hr.) | Rapid Arabinose- Fermentation (within 24 hr.) | Agglutination with B.-Paratyphosus-B No. 12 Serum | |
|--------|---|--|--|--|
| | | | Before Saturation | After Saturation with Suipestifer 167 |
| 62 | + | + | 5000 | 2000 |
| 63 | — | — | 500 | <250 |
| 114 | — | — | 500 | <250 |
| 115 | + | + | 5000 | 2000 |
| 118 | — | — | <250 | <250 |
| 132 | — | — | <250 | <250 |
| 133 | — | — | 5000 | <250 |
| 160 | — | — | <250 | <250 |
| 161 | + | + | 5000 | 2000 |
| 162 | — | — | 2000 | <250 |
| 163 | — | — | 5000 | <250 |
| 165 | — | — | 5000 | <250 |
| 167 | — | — | 5000 | <250 |
| 168 | — | — | 5000 | <250 |
| 169 | — | — | 5000 | <250 |
| 170 | — | — | <250 | <250 |
| 171 | — | — | 500 | <250 |
| 172 | — | — | 1000 | <250 |
| 173 | — | — | <250 | <250 |
| 174 | — | — | <250 | <250 |
| 175 | — | — | — | <250 |
| 177 | — | — | 1000 | <250 |
| 178 | — | — | 1000 | <250 |
| 234 | — | — | 2000 | <250 |

TABLE 13
B.-SUIPESTIFER (167) SERUM (TITER, 1:2000)

| Strains of Human Origin | | | | | Strains of Porcine Origin | | | | |
|-------------------------|-------|-------|--------|--------|---------------------------|-------|-------|--------|--------|
| No. | 1:250 | 1:500 | 1:1000 | 1:2000 | No. | 1:250 | 1:500 | 1:1000 | 1:2000 |
| 2 | + | 0 | 0 | 0 | 62 | tr | 0 | 0 | 0 |
| 5 | 0 | 0 | 0 | 0 | 63 | +++ | ++ | + | tr |
| 8 | 0 | 0 | 0 | 0 | 114 | +++ | ++ | + | tr |
| 12 | 0 | 0 | 0 | 0 | 115 | tr | 0 | 0 | 0 |
| 47 | 0 | 0 | 0 | 0 | 132 | +++ | ++ | + | tr |
| 130 | + | tr | 0 | 0 | 133 | +++ | ++ | tr | 0 |
| 149 | tr | 0 | 0 | 0 | 160 | +++ | ++ | + | tr |
| 150 | 0 | 0 | 0 | 0 | 161 | tr | 0 | 0 | 0 |
| 151 | 0 | 0 | 0 | 0 | 162 | +++ | ++ | + | tr |
| 152 | 0 | 0 | 0 | 0 | 163 | +++ | ++ | + | tr |
| 179 | tr | 0 | 0 | 0 | 165 | +++ | ++ | + | tr |
| 180 | tr | 0 | 0 | 0 | 167 | +++ | ++ | + | tr |
| 185 | 0 | 0 | 0 | 0 | 168 | +++ | ++ | + | tr |
| 202 | 0 | 0 | 0 | 0 | 169 | 0 | 0 | 0 | 0 |
| 203 | 0 | 0 | 0 | 0 | 170 | +++ | ++ | + | tr |
| 209 | 0 | 0 | 0 | 0 | 171 | +++ | ++ | + | tr |
| 210 | 0 | 0 | 0 | 0 | 172 | +++ | ++ | + | tr |
| 211 | 0 | 0 | 0 | 0 | 173 | +++ | ++ | + | tr |
| 221 | 0 | 0 | 0 | 0 | 174 | ++ | ++ | ++ | ++ |
| | | | | | 175 | 0 | 0 | 0 | 0 |
| | | | | | 177 | +++ | ++ | + | tr |
| | | | | | 178 | +++ | ++ | + | tr |
| | | | | | 234 | +++ | ++ | + | tr |

+++ = complete
 ++ = marked
 + = slight
 tr = trace
 0 = none

SUMMARY AND CONCLUSIONS

The cultures of bacilli that I have examined, belonging to the paratyphoid-enteritidis group, fall for the most part into 4 subdivisions.

These may be characterized provisionally as of the following types:

(a) *B. paratyphosus* A, (b) *B. paratyphosus* B, (c) *B. suipestifer*, and (d) *B. enteritidis*.

(a) The *B. paratyphosus*-A strains are all from human sources. They attack arabinose quickly and dulcitol slowly; they do not ferment xylose. They produce alkali in litmus milk, but produce it very slowly so that the initial acidity caused by all members of this group persists for a long time and the color of the control tube is rarely regained in less than 2 weeks. A distinct alkaline reaction, however, has been observed in all cultures at the end of 30 days, usually earlier. The strains possessing these characters (20 in number) form a homogeneous agglutinative group.

(b) The *B. paratyphosus*-B strains (23) attack arabinose, dulcitol, and xylose rapidly, generating gas and acid within 24 hours. They produce alkali quickly in litmus milk, so that a definite blue color is observed usually within 4 to 5 days. Altho differences occur in the speed of alkali-formation, all the slowest alkali-producing strains of this type that have come under my observation have been in advance of the most rapid alkali-producers of the A type. They are all alike in agglutinative characters. The great majority of the cultures of this type are from human paratyphoid infections. A few cultures of porcine origin also possess these characters.

(c) The *B. suipestifer* strains ferment xylose, but attack arabinose and dulcitol slowly or not at all. None of the typical members of this group produce gas in arabinose or dulcitol within 24 hours, in this respect differing from the *B. paratyphosus*-B strains. They also differ from the latter in their agglutinative characters, tho this difference in many cases is manifested only on the application of absorption tests. The *suipestifer* cultures with these characters comprise strains isolated from affected animals in Iowa, Michigan, and Kentucky, as well as stock strains presumably from Maryland and other parts of the United States. In this series the only two European strains said to be of porcine origin (115 from Kral, 161 from Ostertag) possess characters identical with those of the *B. paratyphosus* type. Two strains of known porcine origin (169 and 175) have changed to the *paratyphosus*-B type since first isolated.

(d) The *B. enteritidis* strains are indistinguishable from the *B. paratyphosus*-B strains by any cultural characters, but constitute a distinct agglutinative group.

The strains considered in this paper may be grouped as follows:

| Number of Strains | Xylose | Arabinose | | Dulcitol | | Agglutination Type | Origin | |
|-------------------|--------|-----------|------------------|----------|------------------|--------------------|--------|---------|
| | | Rapid | Slow or Negative | Rapid | Slow or Negative | | Human | Porcine |
| 20 | — | + | | | + | Para A | + | |
| 28 | + | + | | + | | Para B | + | (23) |
| 20 | + | | + | | + | Suipestifer | | + |
| 6 | + | + | | + | | Enteritidis | + | |

Division into these four types is based primarily on the examination of recently isolated strains with known histories. Old stock cultures that have been in laboratory collections for some years often show variations, irregularities, and 'departures from type.' Some give reactions, biochemical and agglutinative, that they did not give when first isolated. On the other hand, some strains that apparently possessed unusual fermentative reactions when first isolated later show conformity to type (179).

Variants and irregular strains are also met with which are apparently intermediate between the several groups.

A few strains of porcine origin possess the characteristics of the B.-paratyphosus-B type. These, however, are all strains that have been under cultivation for some time. All the freshly isolated strains from swine that I have been able to obtain, and the majority of the old stock cultures said to be of porcine origin are of the B.-suipestifer type as characterized in the foregoing.

A number of strains, particularly some of the older cultures, have shown marked variation since they came into my hands. The history of these variable and irregular strains will be discussed in a subsequent paper.

Some of the difficulty experienced by previous observers in the differentiation of the B.-paratyphosus-B and B.-suipestifer types has been probably due to the existence of stock cultures labeled in one way or the other but possessing the cultural and agglutinative character of the opposite type. The extent to which transformation of one 'type' into the other occurs under the ordinary conditions of laboratory cultivation is a matter for further investigation.

If the distinction between the freshly isolated cultures of the B.-paratyphosus-B and B.-suipestifer types here indicated is borne out by further studies, it will establish a basis for the tentative classification of this group. No worker with these bacilli, however, can fail to take into account the large amount of variation and the probable transformation of one type into another, especially under the conditions of artificial cultivation.

B. PARATYPHOSUS A

| Strain | Labeled | Received from | Date |
|--------|---------------------------|----------------------------------|------------|
| 3 | B. paratyphosus..... | Dr. L. O. Scott..... | Feb., 1904 |
| 4 | B. paratyphosus 16A..... | Amer. Museum Natural History.... | Aug., 1912 |
| 9 | B. paratyphosus 294a..... | Amer. Museum Natural History.... | |
| 11 | B. paratyphosus 322A | | |
| (48) | B. paratyphosus a..... | Dr. C. J. Hunt | |
| 131 | B. paratyphosus a..... | Dr. Wm. Litterer | |
| 158 | B. paratyphosus a..... | Dr. M. M. Canavan..... | |
| 188 | Paratyphoid 5..... | Dr. C. L. Cole..... | May, 1916 |
| 191 | S-n A..... | Dr. C. L. Cole..... | May, 1916 |
| 198 | Para A..... | Dr. C. L. Cole..... | June, 1916 |
| 212 | Paratyphosus A H-e..... | Dr. H. Zinsser | Nov., 1916 |
| 213 | Paratyphosus A E-n..... | | |
| 214 | Paratyphosus A S-n..... | | |
| 215 | Paratyphosus A Y-e..... | | |
| 216 | Paratyphosus A R-n..... | | |
| 217 | Paratyphosus A B-w..... | | |
| 218 | Paratyphosus A S-h..... | | |
| 219 | Paratyphosus A C-l..... | | |
| 220 | Paratyphosus A No. 8..... | Prof. E. J. McWeeney..... | Dec., 1916 |
| 230 | Paratyphosus A No. 9..... | Prof. E. J. McWeeney..... | Dec., 1916 |

B. PARATYPHOSUS B

| | | | |
|-----|-----------------------------|--------------------------------------|------|
| 2 | B. paratyphosus 22B..... | Amer. Museum of Natural History..... | |
| 5 | B. paratyphosus Buxton..... | Boston Board of Health..... | |
| 8 | B. paratyphosus..... | Dr. Theobald Smith..... | 1902 |
| 12 | B. paratyphosus..... | | 1912 |
| 47 | B. paratyphosus B..... | Dr. C. J. Hunt..... | 1913 |
| 130 | B. paratyphosus B..... | Dr. Wm. Litterer..... | 1914 |

B. PARATYPHOSUS A—Continued

| Isolated from | Date | Reference | Remarks |
|--|-------------|---|--|
| | | Jour. Infect. Dis., 1904, 1, p. 72 | |
| Blood | 1907 | | Received by Amer. Museum of Natural History from Rocke- feller Institute, Jan., 1911 Received by Amer. Museum of Natural History from Hy- giene Laboratory, Washing- ton |
| Murray case, paraty- phoid epidemic, Boston State Hospital, 1910 | Nov., 1914 | Boston Med. and Surg. Jour., 1914, 171, p. 545 | |
| Sergeant K., Band, 28th Infantry, Mission, Tex. | April, 1916 | | |
| Blood | May, 1916 | Personal communi- cation | |
| Z., Mission, Texas..... | | Personal communi- cation | |
| Feces..... | | Weekly Bull., New York City Health Dept., Oct. 14, 1916 | |
| Stool of paratyphoid case | Aug., 1916 | Personal communi- cation | Stock culture, Lister Institute |

B. PARATYPHOSUS B—Continued

| | | | |
|--|-------|--|--|
| | | | Stock culture from Rockefeller Institute |
| | | | Two other cultures, from other laboratories but similarly labeled, gave identical reac- tions |
| Human gallbladder | 1899 | | |
| Human blood in para- typhoid fever | 1912 | Irons and Jordan, Jour. Infect. Dis., 1915, 17, p. 234 | Identical with 14 other strains isolated from gallbladder and feces of same patient |
| Feces; water-borne epi- demic of paratyphoid fever | 1911 | Arch. Int. Med., 1913, 12, p. 64 | |
| Human blood, paraty- phoid fever | 1913 | Personal communi- cation | Isolated from blood 2 days be- fore case terminated fatally |

B. PARATYPHOSUS B—Continued

| Strain | Labeled | Received from | Date |
|--------|--------------------------------------|----------------------------|------------|
| 149 | B. paratyphosus B E3..... | Dr. W. G. Savage..... | 1914 |
| 150 | B. paratyphosus W-s..... | Dr. W. G. Savage..... | 1914 |
| 151 | B. suipestifer Chesterfield..... | Dr. W. G. Savage..... | 1914 |
| 152 | B. suipestifer M-w..... | Dr. W. G. Savage..... | 1914 |
| 179 | B. paratyphosus Put-In-Bay..... | Dr. G. H. Robinson..... | Nov., 1915 |
| 180 | B. paratyphosus W-y..... | Dr. H. S. Bernstein..... | Feb., 1916 |
| 185 | B. paratyphosus B-y..... | Dr. C. L. Cole..... | May, 1916 |
| 202 | B. paratyphosus B VII1..... | Dr. J. G. Cumming..... | June, 1916 |
| 203 | B. paratyphosus B IX..... | Dr. J. G. Cumming..... | June, 1916 |
| 209 | B. paratyphosus B..... | Major Siler, U. S. A. | Oct., 1916 |
| 210 | B. paratyphosus B..... | Major Siler, U. S. A. | Oct., 1916 |
| 211 | B. paratyphosus B..... | Major Siler, U. S. A. | Oct., 1916 |
| 221 | B. paratyphosus B 338..... | Dr. A. B. Wadsworth..... | Nov. 1916 |
| 222 | "B. Aertrycke No. 1, Strain H".... | Prof. E. J. McWeeney..... | Dec., 1916 |
| 223 | B. Aertrycke No. 2..... | Prof. E. J. McWeeney..... | Dec., 1916 |
| 224 | B. paratyphosus B. No. 3, Strain R-d | Prof. E. J. McWeeney..... | Dec., 1916 |
| 225 | B. paratyphosus B, No. 4..... | Prof. E. J. McWeeney..... | Dec., 1916 |

B. SUIPESTIFER

| | | | |
|-----|-------------------------------------|---------------------------------------|-------------|
| 62 | B. cholera-suis III..... | Johns Hopkins Pathological Laboratory | Oct., 1902 |
| 63 | B. cholera-suis IV "1899 Maryland" | Dr. Theobald Smith..... | Oct., 1902 |
| 114 | Bacillus of hog cholera No. 049.... | Dr. W. E. King..... | April, 1914 |

B. PARATYPHOSUS B—*Continued*

| Isolated from | Date | Reference | Remarks |
|---|-------------|--|--|
| Feces of case of paratyphoid fever | 1908 | Rep. of Med. Officer to Local Govt. Bd., 1908-9, p. 316. Strain (E) Case 1, p. 324 | |
| Feces of case of paratyphoid fever (fatal) | 1908 | Like 149, strain (w) Case 4, p. 325 | |
| Food poisoning cases, Chesterfield, England | 1911 | Peck and Thomson, Special Report on Outbreak of Food Poisoning in Chesterfield, 1911 | Obtained by Dr. Savage direct from Dr. Thomson |
| Organs of fatal food poisoning cases | 1908 | Savage and Gunson, Jour. Hyg., 1908, 8, p. 601 | Savage regards this organism as a suipestifer: "the brawn was certainly infected before cooking" |
| Feces, Put-In-Bay epidemic | 1913 | Jour. Infect. Dis., 1915, 16, p. 448 | |
| Pie mixture, food poisoning outbreak, Westerly, R. I. | 1915 | Jour. Amer. Med. Assn., 1916, 66, p. 167 | |
| Lymph-gland infection in man | | Jour. Infect. Dis., 1916, 18, p. 349 | |
| Human gallbladder at autopsy | Oct., 1915 | Personal communication | "Almost pure culture in gallbladder" |
| Human case of paratyphoid in San Francisco | Dec., 1914 | Personal communication | |
| Blood, case of paratyphoid in San Antonio, Texas | July, 1916 | Personal communication | |
| Blood, case of paratyphoid in San Antonio, Texas | Aug., 1916 | Personal communication | |
| Feces, case of paratyphoid in San Antonio, Texas | Aug., 1916 | Personal communication | |
| Feces of paratyphoid-carrier | April, 1916 | Monthly Bull. N.Y. State Dept. of Health, Oct., 1916 p. 252 | |
| Intestine of fatal case of gastro-enteritis, possibly due to food-poisoning | Sept., 1915 | Brit. Med. Jour., Sept. 30, 1916 | |
| Stock culture from Lister Institute, London | | | Obtained by Prof. McWeeney from Lister Institute "some years ago" |
| Fatal case of paratyphoid fever | 1908 | Jour. Hyg., 1911, 11, p. 68 | |
| Feces, case of paratyphoid | 1916 | Personal communication | |

B. SUIPESTIFER—*Continued*

| | | | |
|-------|-------|---|---|
| | | | Obtained by Johns Hopkins "from the laboratory of the Bureau of Animal Industry, Washington, D. C. Since 1897 it has been in our own stock and perhaps some few years longer" |
| | | Smith and Reagh, Jour. Med. Research, 1903, 4, p. 272 | Regarded as a typical hog-cholera bacillus (Hog cholera a, T. Smith) |
| | | | Received originally from Dr. T. Smith. "Passed several times through hogs" |

B. SUIPESTIFER—Continued

| Strain | Labeled | Received from | Date |
|--------|-------------------------------------|-------------------------|-------------|
| 115 | Bacillus of hog cholera No. 050.... | Dr. W. E. King..... | April, 1914 |
| 118 | Bacillus of hog cholera No. 053.... | Dr. W. E. King..... | April, 1914 |
| 132 | B. cholera-suis B..... | Dr. Marion Dorset..... | May, 1914 |
| 133 | B. cholera-suis C..... | Dr. Marion Dorset..... | May, 1914 |
| 134 | B. cholera-suis..... | Dr. Marion Dorset..... | May, 1914 |
| 160 | B. cholera-suis No. 17008..... | Dr. C. W. Brown..... | June, 1915 |
| 161 | B. cholera-suis No. 17003..... | Dr. C. W. Brown..... | June, 1915 |
| 162 | B. cholera-suis No. 17009..... | Dr. C. W. Brown..... | June, 1915 |
| 163 | B. cholera-suis No. 17001..... | Dr. C. W. Brown..... | June, 1915 |
| 165 | B. suipestifer A..... | Dr. D. J. Healy..... | July, 1915 |
| 167 | B. suipestifer 1..... | Dr. R. E. Buchanan..... | June, 1915 |
| 168 | B. suipestifer 3..... | Dr. R. E. Buchanan..... | June, 1915 |
| 169 | B. suipestifer 7..... | Dr. R. E. Buchanan..... | June, 1915 |
| 170 | B. suipestifer 8..... | Dr. R. E. Buchanan..... | June, 1915 |
| 171 | B. suipestifer 14..... | Dr. R. E. Buchanan..... | June, 1915 |
| 172 | B. suipestifer 18..... | Dr. R. E. Buchanan..... | June, 1915 |
| 173 | B. suipestifer 27..... | Dr. R. E. Buchanan..... | June, 1915 |
| 174 | B. suipestifer 33..... | Dr. R. E. Buchanan..... | June, 1915 |
| 175 | B. suipestifer 39..... | Dr. R. E. Buchanan..... | June, 1915 |
| 177 | B. suipestifer 51..... | Dr. R. E. Buchanan..... | June, 1915 |
| 178 | B. suipestifer 55..... | Dr. R. E. Buchanan..... | June, 1915 |
| 234 | B. suipestifer..... | Prof. D. J. Healy..... | Jan., 1917 |

B. ENTERITIDIS

| | | | |
|-----|--------------------------------|---------------------------------------|------------|
| 50 | B. enteritidis Gärtner 18..... | Amer. Museum Natural History.... | 1913 |
| 51 | B. enteritidis Gärtner..... | University of Chicago..... | |
| 52 | B. enteritidis II Gärtner..... | Johns Hopkins Pathological Laboratory | Oct., 1902 |
| 53 | B. enteritidis..... | Dr. C. J. Hunt..... | Oct., 1913 |
| 206 | B. enteritidis P-r VI..... | Dr. J. G. Cumming..... | June, 1916 |
| 228 | B. enteritidis Limerick..... | Prof. E. J. McWeeney..... | Dec., 1916 |

B. SUIPESTIFER—Continued

| Isolated from | Date | Reference | Remarks |
|--|------------------|---|---|
| | | | Received originally from Kral's collection |
| Spleen of hog..... | Jan., 1903 | | Isolated by Dr. Boxmeyer |
| Hog inoculated with hog-cholera virus | Jan., 1908 | | Isolated from hog inoculated with "Hemstock virus," a strain of hog-cholera virus obtained from a typical outbreak of hog cholera in Iowa |
| Guinea-pig inoculated with hog-cholera virus | 1904 | Bull. 72, Bur. An. Ind., pp. 6, 43 | Virus known as "Herd 1 disease" |
| Spleen of a pig dying from "acute hog-cholera" | 1899 | 18th Ann. Rep., Bur. An. Ind., 1901, p. 566 | |
| Hog, epidemic of hog cholera in Michigan | Before 1913 | | Nos. 160, 162, and 163 were isolated from affected animals in epidemics of hog cholera in different localities in Michigan |
| | | | Received originally from Dr. Ostertag |
| Hog, epidemic of hog cholera in Michigan | Before 1913 | | See remarks on 160 |
| Hog, epidemic of hog cholera in Michigan | Before 1913 | | See remarks on 160 |
| Spleen of hog acutely ill with hog cholera | Aug., 1914 | | |
| Spleen, hog | 1915 | | |
| Spleen, hog | | | |
| Lung, hog | | | |
| Spleen, hog | | | |
| Lung, hog | "Spring" of 1915 | | 167-178 inclusive: From hogs slaughtered for virus at serum plant, Ames, Iowa. All showed typical postmortem lesions of hog cholera |
| Spleen, hog | | | |
| Lung, hog | | | |
| Spleen, hog | | | |
| Lung, hog | 1915 | | |
| Lung, hog | | | |
| Intestine, hog | Nov., 1916 | | |
| Spleen, hog | | | |

B. ENTERITIDIS—Continued

| | | | |
|--|------------|------------------------------------|---|
| | | | Originally from the collection of Rockefeller Institute |
| | | | Source unknown. Stock culture |
| | | | "From the laboratory of Dr. Herbert Durham, University of Cambridge, England, in 1900. It is probably much older" |
| Feces; "Epidemic of paratyphoid B infection" | Dec., 1911 | Personal communication | "Agglutinins in sera for this organism and for B. paratyphoid B" |
| Stool of man who was attending the calves during epidemic of enteritidis | Dec., 1914 | | "Agglutinates 1-10,000. Typical sugar reactions" |
| Organs of a fatal case of food-poisoning | 1908 | Brit. Med. Jour., 1900, 1, p. 1171 | |